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Use of Laboratory Animal Models in Investigating Emphysema and Cigarette Smoking in Humans[1]

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To increase our understanding of biological systems, the first step is to make an observation at the level of organization that we wish to understand. It has yet to be conclusively shown that any laboratory animal has contracted emphysema solely as a result of exposure to cigarette smoke. Available mechanism-of-response studies at the tissue, cellular, or biochemical level of organization are not useful in elucidating the relationship between emphysema and exposure to cigarette smoke without an animal model for the disease. If progress in understanding the etiology of emphysema from cigarette smoking is judged to be important to the scientific and regulatory communities, the highest priority should be given to discovering a suitable animal model.

INTRODUCTION

Animal models have made a significant contribution to our understanding of normal and diseased human pulmonary physiology (O'Neill and Raub, 1984; National Research Council, 1989). Both the U.S. Surgeon General (1984) and the National Research Council (1988) have reported that emphysema has not been demonstrated in whole animals or animal populations exposed to cigarette smoke. However, several possible mechanisms for the development of emphysema from exposure to cigarette smoke in humans have been proposed using laboratory animal-derived data. Among them are the protease–antiprotease mechanism (trypsin–antitrypsin), the presence or formation of oxidant mechanism, and the pulmonary macrophage-releasing protease (lysosomal hydrolase) mechanism (Karlinsky and Snider, 1978; Janoff *et al.*, 1979; U.S. Surgeon General, 1984; Riley and Kerr, 1985; Stockley, 1987).

Because there is no study that shows that cigarette smoke exposure causes emphysema in a laboratory animal model, the mechanism(s) that might be operative in the development of emphysema from cigarette smoke exposure cannot be predicted. The observations made in the studies designed to elucidate mechanisms cannot be used to support the reported epidemiological link between emphysema and cigarette smoking in humans (U.S. Surgeon General, 1984).

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To support this thesis we discuss the appropriate experimental approach that toxicologists must undertake to elucidate mechanism(s). We also review the literature on the search for an animal model linking cigarette smoking and emphysema. [Neither the U.S. Surgeon General's report (1984) nor the National Research Council's report (1988) includes a review of the literature on whole-animal studies.]

EXPERIMENTAL APPROACH

To increase our understanding of biological systems in general, we must first make an observation at the level of organization that we wish to understand. From this principle of experimental procedure it follows that to understand lung disease using an animal model, the first observation must be that disease occurs in the lungs of whole animals (the level of organization of interest). It also follows that measurements of abnormal lung cellular or biochemical responses (a lower level of organization) cannot be used to predict that the whole lung will become diseased. For example, Kleinerman and Ip (1988) have found that the biochemical factors currently thought to control the development of emphysema are adaptive responses in the Syrian hamster that serve to protect the lung tissue from progressive injury. This understanding was reached only after two chronic inhalation studies with the Syrian hamster demonstrated that prolonged NO_2 exposure caused mild emphysema which did not progress.

Interestingly, this principle of experimental design became an issue in the ecological sciences in the 1970s and was elevated to the status of paradigm by students of the population biologist F. E. J. Fry (Kerr, 1976). At that time, the criticism was made that many of the supposed properties of ecosystems (stability, diversity, trophic levels) might not exist because they were formulated from measurements of communities and populations (lower levels of organization) within particular ecosystems (the higher level of organization).

Based on this principle of experimental design, the first task in the discovery of a link between cigarette smoke and emphysema is to define the conditions under which an animal species would develop emphysema from cigarette smoke exposure. Because emphysema is a disorder of anatomy (U.S. Surgeon General, 1984) characterized by abnormal permanent enlargement of the air spaces distal to the terminal bronchioles, accompanied by destruction of the alveolar walls (Huber *et al.*, 1981), anatomic evidence is the highest diagnostic priority (Karlinsky and Snider, 1978). Microscopic examination of histological sections to find and quantify destruction and measurements to determine airspace size enlargement are common techniques used to define emphysema. Secondary evidence in most cases includes examination of lung functions such as compliance (decreased elastic recoil) with accompanying changes in lung volume (Karlinsky and Snider, 1978).

Under the exact conditions in the whole-animal study and the particular species already studied, further sets of experiments using lung tissue (Hoidal and Niewoehner, 1982), lung cells (Flint *et al.*, 1971; Matulionis and Traurig, 1977; Coggins *et al.*, 1980), and lung biochemical functions (Janoff *et al.*, 1979; Osman *et al.*, 1982) in living animals followed by excised lung preparations (Walter and Walter, 1982) and *in vitro* tissue (Hamosh *et al.*, 1979; Laurent *et al.*, 1983) studies could then be undertaken.

THE SEARCH FOR A SUITABLE LABORATORY ANIMAL MODEL

Holland *et al.* (1963) were the first group to report emphysema in laboratory animals exposed to cigarette smoke. They reported that focal emphysema developed earlier, more frequently, and more diffusely in smoke-exposed rabbits than in controls. Seven of the control rabbits and five of the smoke-exposed animals were reported as having focal emphysema. Eleven smoke-exposed rabbits and one control group rabbit had "generalized" emphysema. Emphysema was described as the cause of death for four of the smoke-exposed rabbits. However, no definition was given for emphysema and no quantitative measurements were presented to support their observations. Georgadze and Krasnyanskaya (1967) reported that rabbits exposed to cigarette smoke for 3 years developed bronchitis and bronchiolitis, pneumonia, and emphysema, also with a similar lack of supporting data.

The first report of cigarette-induced emphysema in dogs was by Rockey and Speer (1966). They reported one dog with emphysema in a group of 8 beagles exposed to nonfiltered cigarette smoke through tracheal fenestration and one dog with emphysema in a group of five dogs exposed to filtered cigarette smoke through the nostrils. Evidence presented for emphysema was a single photomicrograph from a single histological section from one dog which claims to show dilation of alveolar ducts and alveoli with some alveoli membranes starting to rupture.

Hernandez *et al.* (1966) exposed 15 adult greyhounds, recently retired from dog racing, to cigarette smoke. They describe "grossly damaged" areas of lung parenchyma with inflammation of bronchioles and small bronchi, alveolar inflammation, and alveolar disruption that appeared to be greater in severity in dogs exposed to cigarettes longer than 1 year. However, the authors did not conclude that the observed alveolar rupture was caused by the cigarette smoke alone, but that it could have been caused by other "infective and/or irritative factors."

Ten beagle dogs were exposed to cigarette smoke for more than 1 year by Auerbach *et al.* (1967). They reported rupture of alveolar septa, fibrous thickening of alveolar septa, and padlike attachments to alveolar septa. In a second study, reported by Hammond *et al.* (1970), four groups of dogs were exposed through tubing inserted into the trachea to smoke from filtered and unfiltered cigarettes. Emphysema was defined as rupture or destruction of alveolar septa and was divided into four grades. The number of dogs with higher grades of emphysema increased with time and with increasing doses of tar and nicotine estimated to be received by the dogs. Frasca *et al.* (1971) examined with an electron microscope lung sections described in the Hammond *et al.* (1970) report as the most emphysematous. Frasca *et al.* observed alveolar thickening due to the presence of large amounts of collagen and a reduced number of capillaries. Also observed were large numbers of macrophages in the alveolar spaces. Carter *et al.* (1976) confirmed the presence of gaps in alveolar walls and enlarging airspaces in lung sections from the same lungs using the scanning electron microscope.

These studies have been criticized for several reasons. Exposing the dogs to cigarette smoke through tracheal tubes was considered to be highly stressful. There was no sham-exposed control group. The dogs were also reported to have various types of lung infection which was a confounding factor (Binns, 1975). Park *et al.* (1977) exposed beagle dogs to cigarette smoke for 1 year by use of a face mask which forced the dog to breathe through the mouth. Morphometry of bronchiolar size distribution,

volume proportion of parenchymal structure, and alveolar surface area were not observed to be affected by cigarette smoke. There were also no observed effects on pulmonary function including diffusion capacity for carbon monoxide, functional residual capacity, residual volume, or volume of inflation that could be attributed to cigarette smoke.

According to Zwicker *et al.* (1978), of 12 dogs exposed to various levels of cigarette smoke for 5 months, 3 were reported to have mild to moderate alveolar emphysema. One dog in the control group was also reported to have emphysema. The method for judging the severity of emphysema was not presented.

Dalbey *et al.* (1980) exposed F344 rats to cigarette smoke for more than $2\frac{1}{2}$ years. They reported "no evidence" for emphysema but did not define it or present evidence to support their observation. Heckman and Dalbey (1982), reporting on the same group of rats, documented morphometric measurements that showed significant airspace enlargement in rats exposed to 10 cigarettes per day. However, this exposure level led to unacceptably high mortality. No reason for the high mortality was given.

Wehner *et al.* (1981) measured alveolar chord length and systal chord length to observe potential emphysematous changes in F344 rats exposed to cigarette smoke for 2 years. Dilated acini and alveoli were reported as generally present in smoke-exposed rats. One rat had a small area of cavitation in the alveolar parenchyma. No statistical differences were observed between smoke-exposed groups and the control group.

Huber *et al.* (1981) exposed 300 pathogen-free albino rats to cigarette smoke for 185 days. Morphometric measurements were made using light microscope fields from the lungs of 10 animals chosen randomly from each of a group of 16 smoke-exposed, 16 sham-exposed, and 16 untreated animals. Two-hundred and fifty microscopic fields from each group, representing individual and measurement variance, were examined. Particulate dose to the lungs was defined using tracer in the tobacco. Gas quasi-static volume–pressure studies *in situ* and saline volume–pressure studies on excised lungs were also conducted on both exposed and control groups.

In the smoke-exposed animals, alveolar surface area was reduced 12% and pulmonary parenchyma was reduced 21% in the cigarette-exposed animals. Altered elastic properties resulted in higher relative lung volumes and larger total lung capacity at maximum inflation pressure, despite the loss of lung tissue. In addition, the residual volume of trapped gas was increased severalfold in the smoke-exposed lungs, suggesting a reduction in parenchymal airway support structures possibly involving the interstitial elastic tissue of the lung.

Huber *et al.* concluded that their data were suggestive but not conclusive in supporting a hypothesis that cigarette smoke can induce emphysema. They cited three reasons. They could not say that the morphometric observations were not caused by differential growth in the different experimental groups. The animals were not disease free. The damage could not be shown to be permanent.

Snell's mice exposed to cigarette smoke for 12 months or longer were reported to display emphysema or chronic interstitial inflammatory changes (Leuchtenberger and Leuchtenberger, 1970). Again, no data were presented to support this observation.

Mention of emphysema in hamsters chronically exposed to cigarette smoke has been made by Dontenwill *et al.* (1973) and Wehner *et al.* (1974). Dontenwill *et al.* (1973) used two methods to count the number of alveoli per unit of surface area of

lung. No significant differences between smoke-exposed and control animals were detected and it was concluded that no increase in emphysema could be observed in the animals exposed to cigarette smoke. Wehner *et al.* (1974) reported that smoke-exposed animals showed signs of emphysema that were variable in degree within the lungs of animals and between exposed animals. No data or methodology for the determination of emphysema were presented.

Aviado *et al.* (1970) exposed Mendel–Osborne rats and Ito *et al.* (1976) exposed Wistar rats to 10 weeks of cigarette smoke exposure. Aviado and Watanabe (1974) exposed ICR and Swiss mice to 10 weeks of cigarette smoke. All studies used functional lung measurements to detect emphysema. No lung pathology was done. No functional signs of emphysema were reported; however, the methods used and the length of exposure (10 weeks) made it highly unlikely emphysema would have been detected even if it had occurred.

DISCUSSION

Several studies reported the presence of emphysema as a result of exposure to cigarette smoke but did not define their methodology or present data to support how this determination was made. These included Holland *et al.* (1963), Georgadze and Krasnyanskaya (1967) for rabbits, Rockey and Speer (1966) and Zwicker *et al.* (1978) in dogs, Wehner *et al.* (1974) in hamsters, and Leuchtenberger and Leuchtenberger (1970) in mice.

Two other studies, which also did not present data or methodology, concluded the opposite. Dalbey *et al.* (1980) and Heckman and Dalbey (1982) concluded that cigarette smoke did not cause emphysema in chronic studies using rats. Hernandez *et al.* (1966), using dogs, concluded that the observed emphysema could not be attributed to cigarette smoke alone.

Studies having negative results supported by the presentation of morphometric measurements are Dontenwill *et al.* (1973) using hamsters and Wehner *et al.* (1981) using F344 rats. Park *et al.* (1977) made both morphometric and pulmonary function measurements and found no evidence of emphysema in beagle dogs.

The strongest evidence that laboratory animals contract emphysema is found in the series of beagle dog studies reported by Auerbach *et al.* (1967) and Hammond *et al.* (1970) and the supporting microscopic work of Frasca *et al.* (1971) and Carter *et al.* (1976). Morphometric analysis appears to show that the dogs in the cigarette-exposed group contracted emphysema and that it was more severe in the groups exposed to cigarette smoke than in the control group. In addition, a dose–response relationship was reported to have existed. However, lack of adequate controls makes it impossible to judge the significance of potentially confounding factors such as infections and stresses from the unusual method of exposure.

The study of most interest is that of Huber *et al.* (1981) using albino rats. Almost all conditions were carefully controlled, methodology and results were well documented, and the authors showed complete awareness of the potential confounding factors in carrying out such a study. However, the study was terminated after 6 months and disease-free conditions were not maintained in the laboratory. Despite the fact that both morphometric and pulmonary function measurements indicated emphysema in the smoke-exposed animals, the authors could not conclude that their observations

were a result of exposure to the cigarette smoke. In summary, it has yet to be conclusively shown that any laboratory animal models have successfully produced emphysema solely as a result of exposure to cigarette smoke.

This is not the first time it has been reported that emphysema has not been demonstrated in whole animals exposed to cigarette smoke. According to Binns (1975), "no convincing studies have been published on emphysema in small animals in relation to cigarette smoking." This conclusion was repeated by Huber *et al.* (1981), in Chapter 5 of the U.S. Surgeon General's report on chronic obstructive lung disease (U.S. Surgeon General, 1984), and most recently by the National Research Council (1988). None of these reports emphasized the importance of this deficiency.

What this means is that further inferences concerning the relationship between cigarette smoke and emphysema using current animal models cannot be made at this time. What is needed is a definitive whole-animal study that would eliminate confounding factors such as infection and parasitic load. A complete study would demonstrate the extent to which the observed damage was permanent and not repairable (Huber *et al.*, 1981; Kleinerman and Ip, 1988). Simultaneous dosimetry data would be collected to define the conditions under which the observations were made (Dontenwill *et al.*, 1973; Huber *et al.*, 1981). Careful attention to variance of measurement is important. The study would be designed for more than 6 months' duration. The methods and recommendations described by Huber *et al.* (1981) appear to be the most rigorous and complete in the published literature.

Unfortunately, it is not possible to say that a successful and repeatable animal model of emphysema induced by chronic exposure to cigarette smoke would be without controversy as a model for emphysema in humans, primarily because there is controversy over the current classification system. The animal model will be most readily received if it appears to be similar to severe centrilobular emphysema (U.S. Surgeon General, 1984).

CONCLUSION

To increase our understanding of biological systems, the first step is to make an observation at the level of organization that we wish to understand. It has yet to be conclusively shown that there is a laboratory animal model for emphysema produced solely as a result of exposure to cigarette smoke. Therefore, if progress in understanding the etiology of emphysema from cigarette smoking is judged to be important to the scientific and regulatory communities, the highest priority should be given to carefully designed whole-animal studies that will show emphysema.

Until emphysema can clearly be produced in a laboratory animal model, available mechanism-of-response studies at the tissue, cellular, or biochemical level of organization are not useful in elucidating the relationship between emphysema and exposure to cigarette smoke. Which studies might eventually become useful depends on which animal model is shown to contract emphysema from cigarette smoke.

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